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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/161,680	09/28/1998	UWE BORNSCHEUER	48429	7944

26474 7590 12/10/2002

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EXAMINER	
KERR, KATHLEEN M	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 12/10/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/161,680	Applicant(s) BORNSCHEUER ET AL.	
	Examiner Kathleen M Kerr	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 September 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *12-23* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *12-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Application Status

1. In response to the previous Office action on the merits (Paper No. 20 mailed January 4, 2002), an RCE (Paper N0. 22) and an amendment (Paper No. 23) were filed on June 10, 2002 after a personal interview on June 3, 2002 (Paper No. 21). Applicants also filed a supplemental amendment (Paper No. 25 received on September 30, 2002) in response to a Notice of a non-responsive amendment (Paper No. 24 mailed on August 26, 2002).

Said previous Office action rejected all claims, Claims 1, 2, 4-7, 10, and 11. Applicants' newly filed amendment (Paper No. 23) canceled all previously pending claims and added new claims 12-23. In Paper No. 25, Applicants further amended Claims 12 and 22. Thus, Claims 12-23 are pending in the instant application and will be examined herein.

Priority

2. The instant application is granted the benefit of priority for the foreign application 19743683.8 filed in Germany on October 2, 1997 as requested in the declaration.

Drawings

3. As previously noted, the drawings have been approved by the Draftsmen and are, therefore, entered as formal drawings acceptable for publication upon the identification of allowable subject matter.

Withdrawn - Claim Rejections - 35 U.S.C. § 112

4. Previous rejection of Claims 1, 2, 4-7, and 11 under 35 U.S.C. § 112, second paragraph, as being indefinite for the term "substrate specificity" is withdrawn by virtue of Applicant's cancellation of said claims.

Withdrawn - Claim Rejections - 35 U.S.C. § 102

5. Previous rejection of Claims 1-2 and 4-7 under 35 U.S.C. § 102(b) as being anticipated by Greener *et al.* is withdrawn by virtue of Applicants' cancellation of said claims.

Withdrawn - Claim Rejections - 35 U.S.C. § 103

6. Previous rejection of Claims 1-2, 4-7, and 11 under 35 U.S.C. § 103(a) as being unpatentable over Greener *et al.* in view of Wilks *et al.* is withdrawn by virtue of Applicants' cancellation of said claims.

Issues Newly Presented (all previous issues are withdrawn)

Objections to the Specification

7. The specification is objected to for lacking appropriate sections entitled as follows:

a) Background

b) Summary of the Invention

c) Detailed Description of the Invention

Appropriate correction is required.

8. The specification is objected to for a confusing structure using parentheses. On page 6, line 4, and in several other occurrences throughout the specification, the following structure "altered substrate specificity (= mutations in the enzyme used)" is used and is unclear. Is the phrase in parentheses meant to replace its modified term? It is wholly unclear. Appropriate correction and/or clarification are required.

9. The specification is objected to for being confusing on page 9. In the examples, structures are described. Formula (II) contains an R group that is defined on the left of the insert as CH_2CH_3 when formula (II) 1 is used. The text also refers to formula (II) 3; however, the "3" on the left of the insert does not contain a definition of R. The presence of the "3" here is wholly unclear as is this version of formula (II). Appropriate correction and/or clarification are required.

10. The specification is objected to for being confusing considering the Table I on page 10. The abbreviations throughout the table, like "PS" are wholly unclear. Appropriate correction and/or clarification are required.

Objections to the Claims

11. Claims 12 and 22 are objected to for having an improper form. After item d), the conjunction "and" is required.

Claim Rejections - 35 U.S.C. § 112

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

12. Claims 12-23 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. As noted for the previously pending claims that had been rejected on this basis, the specification loosely defines "substrate specificity" in the following sentence from page 4, lines 1-3: "The alteration in the substrate specificity reduces the K_m or increases the k_{cat}, or both, i.e. the ratio k_{cat}/K_m becomes greater than zero." As the Examiner has previously noted, the art defines substrate specificity by k_{cat}/K_m; the "greater than zero" phrase in the specification is unclear. Moreover, on page 6, the specification seems to equate "altered substrate specificity" simply with "(=mutations in the enzyme used)". This is wholly unclear. Nowhere in the specification can a clear definition of the term "substrate specificity" be found. Thus, its metes and bounds are unclear.

The Examples in the instant specification describe synthesizing a substrate (let's call it substrate A) that the inventors wanted to have an enzyme utilize to produce a particular product, wherein the product is difficult to organically synthesize; the chemical reaction is a lipase or esterase type reaction. Since no known enzyme naturally performs this reaction with substrate A, the inventors subjected an esterase gene to random mutagenesis in the hopes of altering a naturally occurring lipase or esterase to now accept substrate A and catalyze the desired reaction. These mutant esterases were screened for the desired activity. Having all this information, the term "substrate specificity" still is not clearly defined in the specification, particularly with respect to the examples that do not assess the mutant or wild-type enzymes for K_m values, so that the metes and bounds of the generic methods claimed are clear.

13. Claims 12-23 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The term "functional derivative" is wholly unclear. The metes and bounds of the function that must be retained are undefined. Some particular resistance? The mutator strain functionality? Appropriate clarification is required.

14. Claim 20 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The abbreviations "PS" and "AH" appear in the claims without definition therein or in the specification. Thus, the enzyme names are unclear.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

15. Claims 12-23 are rejected under 35 U.S.C. § 112, first paragraph, written description, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Although the genus of functional derivatives of the mutator strain *E. coli* XL1-Red is discussed in the specification, there is no evidence that any representative species of such a large and varied genus was in the possession of the inventors at the time of filing. To satisfy the written description aspect of 35 U.S.C. § 112, first paragraph,

for a claimed genus of molecules, it must be clear that: (1) the identifying characteristics of the claimed molecules have been disclosed, e.g., structure, physical and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or a combination of these; and (2) a representative number of species within the genus must be disclosed. The specification does not disclose any representative species of any of the recited classes of functional derivatives of a mutator strain, with or without identifying characteristics. Therefore, Claims 12-23, as written, fails to satisfy the written description requirement to the full extent of their scope.

16. Claims 12-23 are rejected under 35 U.S.C. § 112, first paragraph, written description, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The instant claims are directed to methods altering an enzyme's substrate specificity using (1) a random mutator strain, (2) a gene for the unmutated enzyme, (3) a new, desired substrate for the enzyme, and (4) a screening procedure.

The Court of Appeals for the Federal Circuit has recently held that a "written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as be structure, formula [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." University of California v. Eli Lilly and Co., 1997 U.S. App. LEXIS 18221, at *23, quoting Fiers v. Revel, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) (bracketed material in original).

Just as the claims at issue in UC v. Lilly defined the invention by the function of the claimed DNA (encoding insulin), the instant claims define the claimed methods using products only by their functional properties. The Court held this sort of functional definition insufficient. "In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly such a formula is normally an adequate description of the claimed genus. In claims to methods using genetic material, however, a generic statement such as 'vertebrate insulin cDNA' or 'mammalian insulin cDNA,' without more, is not an adequate written description of the genus because it does not distinguish the genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus. A definition by function, as we have previously indicated, does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is" in UC v. Lilly, at *24-*25.

The instant claims are drawn to using *any* enzyme and *any* new substrate to produce a new enzyme with altered substrate specificity relative to the original. The specification provides a *single* example of such enzymes and substrates and no correlations between their structures and functions. The field of enzymology is enormous with six major enzyme categories (provided by the Enzyme Commission in the form of E.C. numbers) and numerous subdivisions within each category based on the functionality of each enzyme. For example, how different from the typical

esterase substrate can you get and still practice the claimed method effectively? Is there any correlation between how different the substrate and how many rounds of mutagenesis are necessary to achieve the desired goal? Are there occasions that structurally the method will not work? Considering all these questions, it is clear that the written description of a single example in the instant specification does not adequately describe the genus of "reagents" claimed for use in the methods.

17. Claims 12-23 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for specific examples of the methods proven to achieve their goals, does not reasonably provide enablement for methods using all enzymes, all substrates, and all possible mutator strains. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims. To practice the claimed methods to the full extent of their scope would require undue experimentation.

The factors to be considered in determining whether undue experimentation is required are summarized In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). The Court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (Wands, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (Wands, 8 USPQ2d 1404). The factors to be considered

in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

The Examples in the instant specification describe synthesizing a substrate (let's call it substrate A) that the inventors wanted to have an enzyme utilize to produce a particular product, wherein the product is difficult to organically synthesize; the chemical reaction is a lipase or esterase type reaction. Since no known enzyme naturally performs this reaction with substrate A, the inventors subjected an esterase gene to random mutagenesis in the hopes of altering a naturally occurring lipase or esterase to now accept substrate A and catalyze the desired reaction. These mutant esterases were screened for the desired activity. No guidance is suggested for the use of other enzymes, with other catalytic activities. No guidance is suggested for what sort of substrates can be utilized – how alike to the original substrate they must be. The amount of experimentation to randomly screen for a "novel" enzyme activity is wholly dependent on the type of substrate looking to be used by the "new" enzyme – a substrate very similar to the original is likely to take little experimentation while a substrate unlike the original is unlikely to produce any positive result at all. No guidance as to where to draw this line is offered by the specification as originally filed. The most striking of the Wands factors to be considered is the extreme unpredictability in the claimed methods. It is unclear from the art and the specification which enzymes might facilitate such methods. Some enzymes, as tested by site-directed

mutagenesis, can tolerate little mutation in their active sites and/or substrate binding pockets and still perform their catalytic activities. These would not be useful for the claimed methods. Other enzymes do not have as much know about their structures, and their effectiveness in the claimed methods is wholly unpredictable. For all these reasons, the instant claims are not enabled to the full extent of their scope.

Claim Rejections - 35 U.S.C. § 103

The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

18. Claims 12, 16, and 21 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Wilks *et al.* in view of Greener *et al.* The instant claims are drawn to methods of altering substrate specificity of a hydrolase using an *E. coli* mutator strain, XL1-Red, in an iterative process of random mutagenesis and screening using a substrate not previously utilized by the hydrolase.

Wilks *et al.* generally teach the "alteration of enzyme specificity and catalysis by protein engineering" (see Title). More specifically, Wilks *et al.* teach the broadening of the substrate specificity of lactate dehydrogenase (see page 562-563). "Creation of an enzyme with broad substrate specificity was required for the chiral synthesis of lactates (R-HCOH-COOH) with a wide range of large R-groups. These compounds are intermediate in the synthesis of many

present day and future high-volume single-compound pharmaceuticals." Based on the mouse sequence for LDH, whose encoded enzymes are known to have broader specificities, redesign of the more substrate-stringent *B. stearothermophilus* LDH enzyme is taught. Particular substrates are assayed with the mutant enzyme in the bacteria to determine the effectiveness of the redesign. Wilks *et al.* teach that their results can be achieved by redesign (rational design and construction), as typified in their examples, or random mutagenesis and screening (see page 561, left column). Wilks *et al.* do not teach particular examples of random mutagenesis and screening using hydrolases.

Greener *et al.* teach methods of random mutagenesis of a cloned phosphatase gene using the *E. coli* strain XL1-Red for the purpose of introducing single, random point mutations into said gene, iterating the process for several generations to achieve the appropriate degree of mutation in said gene, and screening for phenotypic variants of said gene in a nonmutator host organism, specifically *E. coli*. The cloned gene specifically used in Greener *et al.* is alkaline phosphatase, which is generally classified in the hydrolase family of enzymes.

It would have been obvious to one of ordinary skill in the art to combine the teachings of Wilks *et al.* and Greener *et al.* to practice a method wherein a mutator strains induce changes in a gene of interest encoding a particular enzyme for the purpose of altering said enzyme's substrate specificity. One would have been motivated to combine the teachings of Wilks *et al.* and Greener *et al.* because Wilks *et al.* discuss the usefulness of novel enzymatic activities in the biosynthesis of therapeutics and, while random mutagenesis is not as predictably effective (see Wilks *et al.* (page 563, right column), it can be performed more easily and with less information about the structure of the enzyme. One would have had a reasonable expectation of success that

the methods taught the combined references would produce an phosphatase with altered substrate specificity because this is, in fact, indicated in the teachings of Greener *et al.* "presumably, these structural gene mutations result in a variant having higher specific activity" (see page 384).

Response to Arguments

19. In response to the previously noted issues of clarity under 35 U.S.C. § 112, second paragraph, concerning the phrase "substrate specificity", Applicants argue that the amended claims address the issues necessary. The Examiner has fully considered this argument, but it is not found persuasive as noted above in the rejected claims.

20. In response to the previously noted issues of art under 35 U.S.C. § 102(b) and 103(a), Applicants arguments have been fully considered but are not deemed persuasive. Applicants argue that the art does not teach all the aspects of the claimed invention. This is not found persuasive because, as is clear from the art rejection set forth above, the art does teach all the aspects of the claimed invention.

Conclusion

21. Claims 12-23 are rejected for the reasons identified in the numbered sections of this Office action. Applicants must respond to the objections/rejections in each of the numbered sections in this Office action to be fully responsive in prosecution.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kathleen M Kerr whose telephone number is (703) 305-1229. The examiner can normally be reached on Monday through Friday, from 8:30am to 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathupura Achutamurthy can be reached on (703) 308-3804. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 872-9306 for regular communications and (703) 872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

KMK
December 9, 2002